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Histopathological Changes of Methanolic Leaf Extract of Diospyros Ferrea (Willd.)Bakh on STZ Induced Diabetic Rat Liver and Pancreatic Tissues.

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ABSTRACT: Histological sections of Liver and Pancreatic tissues were examined under light microscope when treated with the methanolic leaf extract of **Diospyros ferrea** (family:Ebenaceae) has shown a favourable hepatoprotective activity of Streptozotacin (60mg/kg bw) induced diabetic rats on 21st day of experimentation..The daily oral administration of leaf extract of **D.ferrea** (200 & 400mg/kg bw) were tested, the 400mg/kg b w dose of leaf extract brought out significant repair of the hepatic acini and pancreatic islets in the diabetic rats .The liver tissue of diabetic rat exhibited foci of inflammation and necrosis of cells and in the pancreatic tissue cystic degeneration of islet cells along with periductular fibrosis were observed. The leaf extract showed restoration of normal glandular and non-glandular tissue with exception of ductular hyperplasia in pancreatic epithelial cells and also in the pancreas cystic degeneration of islet cells along with periductular fibrosis was observed. Mild peri-biliary infiltration of inflammatory cells and proliferation of bile ductules were exhibited by the liver and liver tissue of diabetic rat exhibited foci of inflammation and necrosis of cells were shown in figures 1&2(a-e) photomicrographs. The glebenclamide (0.5 mg/kg bw)treated diabetic rats revealed that the reduced islets were regenerated to near normal condition in the morphology of pancreatic islets and hepatocytes.Elevated Lipid Peroxidase (LPO:170.16) enzymes, decreased Sodium Oxide Dismutase (SOD:131.83), Catalase (CAT:8.5) and Glutathione (GAT76.0) levels shows that remarkable positive improvement in the hepatocytes and Islets than those of the glebenclamide treated diabetic rats. The rats treated with glebenclamide drug showed is significant (P< 0.001) and the methanolic leaf extract doses of 200mg/kg and 400mg/kg also brought down the ALT and AST enzyme quantities significantly is due to dose dependent and statistically significant at P< 0.001 level.

KeyTerms : Histopathology, Diabetic rats. Pancreas, Liver, Streptozotocin,,Glibenclamide.

I. INTRODUCTION:

Hepatic and pancreatic endocrine tissues play an important role in glucose homeostasis through glycolysis, glycogenesis, gluconeogenesis but liver is affected severely during diabetes. The net glucose uptake by the liver depends on the activities of glucokinase and glucose-6-phosphatase were markedly decreased almost the activity of glucose-6-phosphatas and is doubled in diabetic condition (Vinayagam Ramachandran et al., 2012). As liver is the major site of drug metabolism, hepatotoxic chemicals damage hepatocytes by inducing lipid peroxidation and other oxidative damages (Ilango and Chitra, 2009).

Diospyros ferrea ,widely distributed in Indian evergreen forest of South India,which is the largest genus of Ebenaceae family with 500 species used in traditional medicinal systems. Pullaiah and Sandhya Rani (1999) reported 11 species of Diospyros from Andhra Pradesh. Asolkar et al., (1992) reported the active principle compounds responsible for medicinal properties present in twelve Indian Diospyros species..In majority of them betulinic acid, beta-sitosterol, lupeol, triterpenes, saponins and tannins are reported. Betulinic acid is known to exhibit a variety of biological and medicinal properties (Mansour et al., 2012). Zakaria et al., (1984) commented that the napthoquinones present in genus Diospyros are possibly responsible for antibacterial activities. Many investigators reported that saponins, steroids, tannins and terpenoids possess antibacterial, antiviral, antihelminthic, anti hyperglycemic and anti-inflammatory activities (Malairanjan et al,

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2006; Riever et al., 2009; Rathore et al., 2012). The phytochemical analysis, antidiabetic and anti-inflammatory effects of Diospyros ferrea have not been so far studied by any other investigator,.So I have carried out my research work.

Diospyros ferrea is found in Saseshachala hills of Tirupathi,, Khailas hill of Visakhapatnam and Kondapalli forest ranges of Krishna district in Andhra Pradesh. The wood is considered as black ebony and fruits are edible. The wood is used in the manufacture of furniture, cabinet, in laying leaves, tool handles, finger boards and key of guitars and violins, bobbins and spindles etc. (Vijayalakshmi et al., 2012).The leaves are simple, alternate and distichous about 3-11 by 1.5-7 cm in length. Shape of the leaf is oblong or elliptic or obovate. Apex is acute, base is acute to alternate or slightly rounded. Margin is entire, blade coriaceous, glabrous on both sides. Midrib of the leaf is flat above, primary vein is single, secondary veins are oblique. The mid rib is widely parallel, tertiary veins are oblique. Petiole silky pubescent later glabrescent. Stipule is absent.Flowers white or yellow, 3-merous, male flowers in short cymes, female 10-13, together. Sepals and petals 3 each. Stamens 6, Ovary 3-l ocular, ovule 1 per locule, axile. Berries globose, Yellow brownish red and Orange when ripe.

A branch of Diospyros ferrrea

Diospyros ferrea dried leaf

Recent studies revealed that plant derived natural extracts contain a wide variety of pancreatic α-amylase inhibitors which are of therapeutic importance as oral hypoglycemic agents in diabetes mellitus(Fred-Jaiyesimi & Richard 2009; Sudha et al., 2011; Liozzo et al., 2009, Sudha Ponnusamy et al., 2012). Comparison of the normal and diabetic groups when exposed to D ferrea leaf extract clearly shows destruction of islet cells in diabetic rats as they were irregularly shaped and atrophic, also there was degeneration of the islets cells with varying degree of traversing tiny fibrosepta separating the tissues into compartments in the diabetic rats. The islets of MLEDF treated rats while islets of gibenclamide treated diabetic rats compared to these two groups with the untreated diabetic rats.There was an evidence of recovery of β-cells of the islets, reduced fibrosepta and a more obvious islet pattern with well outlined boundaries but vacuolation was reduced or absent in many

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islets. Also, reduced areas of hemorrhage were observed in the D.ferrea and Gibenclamide treated rats when compared with the diabetic group of rats. This indicates wound healing effects in areas of cellular injury also.

The morphology of the pancreas of MLEDF(methanolic leaf extract of D.ferrea treated diabetic rats revealed remarkable improvement in the islet of Langerhans than those of the Glibenclamide treated diabetic rats. Although, the mode of action of this extract has not been documented, the observed hypoglycemic effects could be due to the combined activity of these compounds demonstrated a possible similarity in their mechanism of action. Decrease in body weight of diabetic rats is due to catabolism of fats and protein (Subash Babu P, Prabhu sreenivasan S and Ignacimuthu S.,2007) was observed and at the end of the experiment, a significant increase in body weight was observed in the groups of animals treated with **Diospyros** and glibenclamide exposed rats attributed to the potent hypoglycaemic activity.

II. MATERIALS AND METHODS:

All the normal, diabetic control and treated animals were sacrificed on 21st day and transverse section of liver and pancreas were prepared by the Paraffin method (Humanson 1979). Histopathological observations were made with light microscope (Gurr 1958). Experimental animal study carried on healthy male Wister rats weighing between 200 to 250 g were housed in clean plastic cages under natural light (12hrs + 12hrs) at room temperature. Animals in all groups were fed normal laboratory feed ad libitum and allowed free access to water. The rats were randomly divided into four groups A, B, C and D of 6 rats each. Diabetes mellitus was induced by a single intraperitoneal injection of STZ (60 mg/kg b w) freshly dissolved in 0.1 M citrate buffer (Rossini AA, Like AA, Appel MC and Williams RM,1978).

After an overnight fast, blood samples were obtained from the retro peritoneal region of the rats and were sacrificed by anesthesia with chloroform and a mid-line incision was made through the anterior abdominal walls. The pancreas is located at the junction of the supra-colic and infra-colic compartments of the abdominal cavity as it extends transversely across the posterior abdominal wall between the duodenum on the right and the hilum of the spleen on the left; the pancreas was isolated from the surrounding organs.

Acute toxicity studies

Acute oral toxicity study was performed as per the, "Organization for Economic Cooperation and Development (OECD)" guidelines. Acute toxicity was determined according to the method of Litchfield and Wilcoxon (1949). Stepwise dose of methanolic leaf extract of Diospyros ferrea (50mg/kg - 2000mg/kg b.w), was administered. Wister rats (200-250 grams weight) were observed individually during the first 30 minutes and periodically during the first 24 hrs with special attention at regular intervals. Daily thereafter no toxic effects and mortality was observed up to 14 days.So the dose of 2000 mg/kg was found to be safe and no toxicity was observed.

Selection of Dose, Experimental design and Collection of blood sample

The LD_{50} cut off value was found to be 2000 mg/kg bw, for the evaluation of anti-diabetic activity, two dose levels were selected i.e., first dose is one-tenth of LD_{50} cut off value and second dose was twice that off one-tenth dose (200 mg/kg and 400 mg/kg. bw)

Experimental design:

Group	Treatment
Group-I	Normal rats
Group-II	Diabetic control rats given streptozotocin (60 mg/kg bw)
Group-III	Diabetic rats given Glebenclamide (0.5mg/kg bw)
Group-IV	Diabetic rats given methanolic leaf extract (200mg/kg bw)
Group-V	Diabetic rats given methanolic leaf extract (400mg/kg bw)

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The blood samples were drawn on 0^{th}, 7^{th}, 14^{th} and 21^{st} day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged (2,500 rpm/10 min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, total protein, Aspartate amino transferase (AST) and Alanine amino transferase (ALT), liver antioxidant enzymes.

Histopathological Experimentation:

All the normal, diabetic control and treated animals were sacrificed on 21^{st} day and transverse section of liver and pancreas were prepared by the Paraffin method (Humanson 1979). Histopathological observations were made with light microscope. Paraffin-embedded sections of pancreas was fixed in Bouin's fluid for 24 hours were cut at 5 µm and stained with hematoxylin and eosin (H&E) and Gomori aldehyde fuchsine for light microscopic examination of the pancreatic islets architecture. The sections were examined under a Leica research microscope (Leitz Wetzlar, Germany) with a Leica EC3 camera attached. Digital photomicrographs of the pancreatic sections were taken at various magnifications.

Reagents :

Ice cold saline, 10% formalin, Alcohol, Paraffin wax, Neutral deparaffinated xylene.

Procedure:

Histopathological studies are carried out by the method of Gurr (1958). At the end of 21 days of experimentation, diabetic and treated animals were sacrificed by cervical decapitation. Pancreas and liver were dissected out, washed in ice-cold saline to remove the blood and were immediately transferred to the fixative of 10% formalin. After the completion of the fixation period, tissues were dehydrated to remove the water. The tissues were dehydrated through passing different percentage of alcohol-water mixture. For stepwise dehydration, the tissues were kept in the progressive alcohol-grades for the following duration; 30% and 50% grades for 5 to 10 hours: in 70% grade for 10 to 12 hours: in 90% grade for about 30 minutes and in absolute alcohol (100%) for 20 minutes. After dehydration, the tissues were embedded in paraffin wax to make it firm for the purpose of section cutting. The thickness of section was adjusted at 6 micron (µ) and then stained with hematoxylin and eosin dye which is mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

III. RESULTS:

Histopathology of liver and pancreas in diabetic rats treated with glebenclamide and D. ferrea leaf extract.

During diabetes the liver and pancreas usually undergo several pathological changes. In the present study STZ –induced diabetic rats were treated with standard drug glebenclamide and two different doses (200 & 400mg /kg b.w) of D.ferrea methanolic leaf extract. These were further subjected to histopathological studies that were carried out in liver and pancreas. The results were interpreted by comparing the anti- diabetic effects of both the standard drug and plant extract with STZ induced diabetic changes.

Liver tissue of healthy normal rat contained normal hepatic lobule architecture and central vein. Hepatocytes appeared normal in portal and periportal regions of liver (figure -1a). In STZ-induced diabetic rats the liver exhibited mild peri-biliary infiltration of inflammatory cells and proliferation of bileduct (figure-1b). The hepatocytes of diabetic rats treated with glebenclamide appeared normal in portal, periportal and central lobular regions (figure-1c). The methanolic leaf extracts (400mg) of D.ferrea plant also showed similar change and resulted in the restorataion of normal hepatic structure, where as in 200mg /kg treatment some foci of inflammation were noticed (figure-1d&e).

The pancreas of healthy rats was with normal acini and normal population of islet cells. The glandular and non-glandular regions ware also normal in healthy rats (figure-2a). In diabetic rats cystic degeneration of non-galndular region and periductular fibrosis were noticed (figure-2b). Glebenclamide treated rats showed restoration of normal glandular and non-galandular cells with exception of ductular hyperplasia in pancreatic epithelalial cells (figure-2c). The histopathological changes in the pancreas of diabetic rats recorded after 21 days of 200mg/kg plant extract treatment showed similarity to the effects of glebenclamide drug (figure-2d).Diabetic rats tratead with 400mg/kg D.ferrea plant extract also showed normal pancreatic cells bearing with the presence of some vacuolar acini in the non-grandular region (figure-2e).

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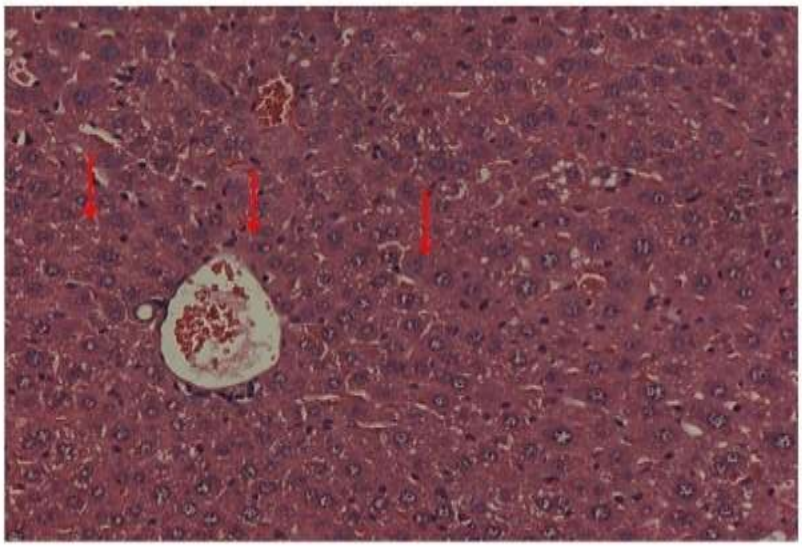
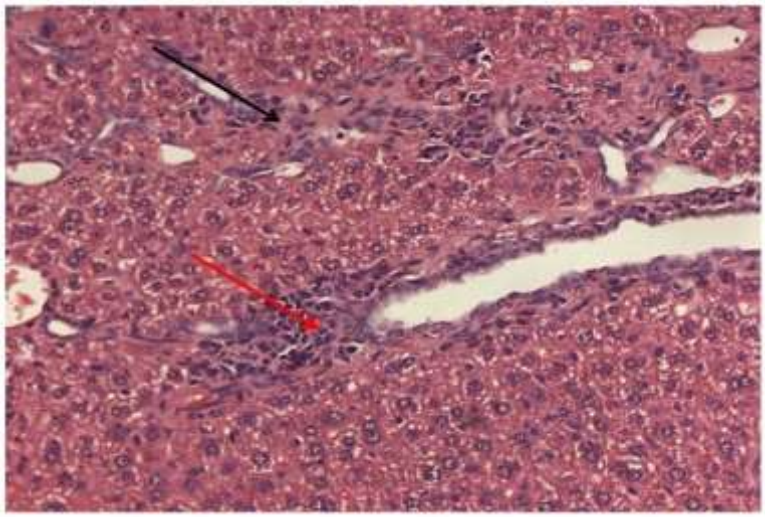
HEPATIC TISSUE

Fig1:(a-e). Photomicrographs showing Histological changes in liver of normal, diabetic and leaf extract treated rats.



a. Normal rats: Hepatocytes appeared normal (red arrow) in portal, periportal region of liver



b. Diabetic control rats: Mild Peri billary infiltration of inflammatory cells (red arrow) Mild proliferation of bile duct are noticed in the liver (black arrow)

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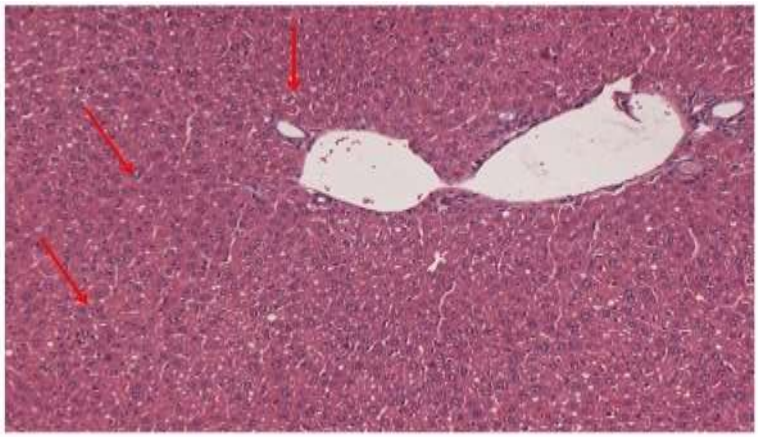
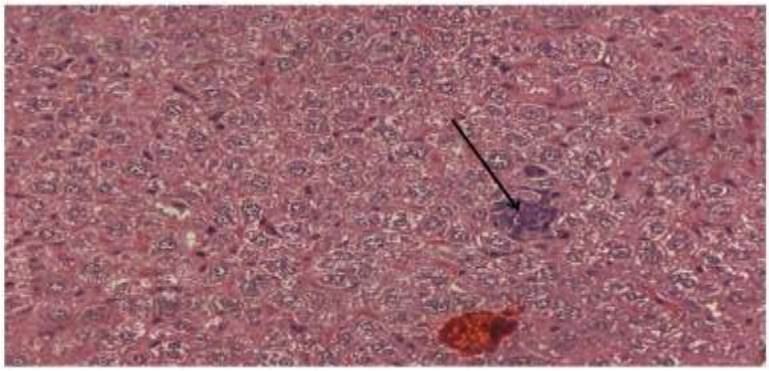
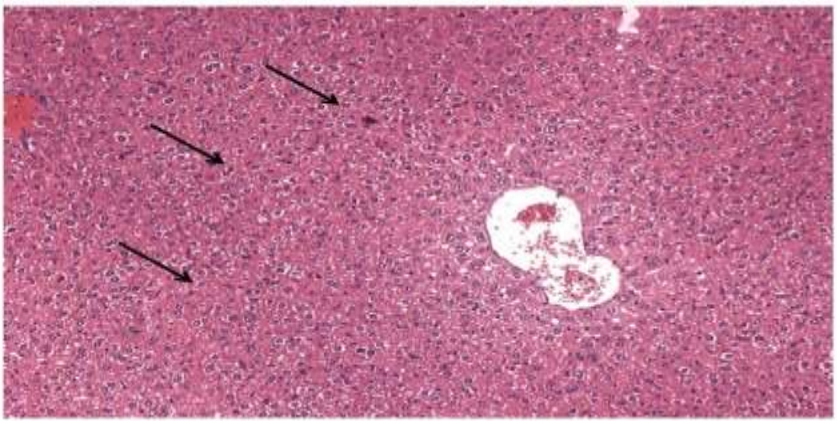


c. Glebenclamide treated rats: Hepatocytes appeared normal in portal, periportal and centri lobular region (red arrow). No necrosis or inflammation noticed in the liver



d. Methanolic leaf extract (200mg/kg b.w) treated rats: Foci of inflammation noticed (arrow)



e Methanolic leaf extract (400mg/kg b.w.) treated rats: Hepatocytes appeared normal (arrow)

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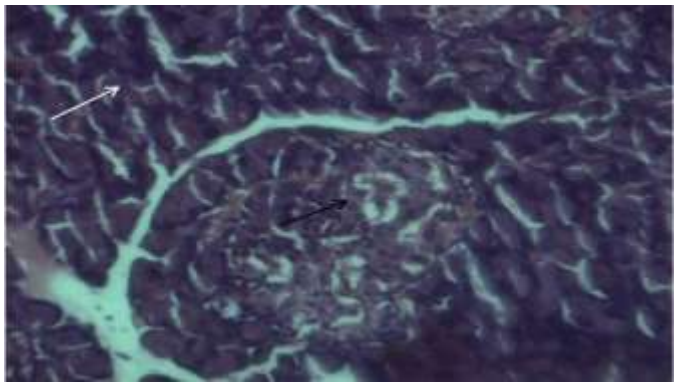
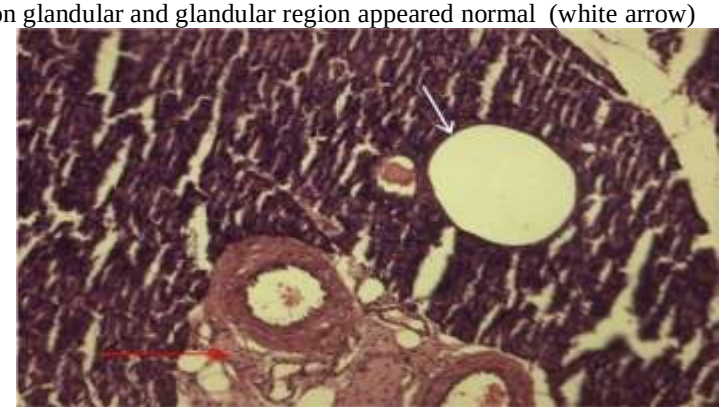
PANCREATIC TISSUE:

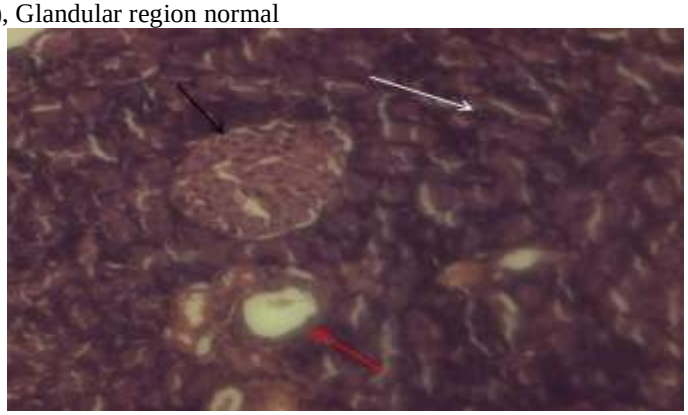
Fig 2: (a-e). Photomicrographs showing Histopathological changes in pancreas of normal rats and methanolic leaf extract treated rats.



a. Normal rats : Non glandular and glandular region appeared normal (white arrow)



b. Diabetic control rats: Cystic degeneration of non glandular region observed (white arrow), Periductular fibrosis (red arrow), Glandular region normal



c. Glebenclamide treated rats : Glandular pancreas with islets cells normal (black arrow), Non glandular region normal (white arrow), Ductular epithelial cells hyperplasia noticed (red arrow)

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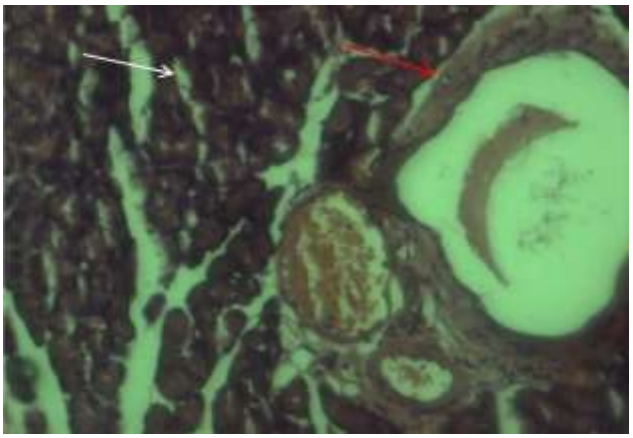
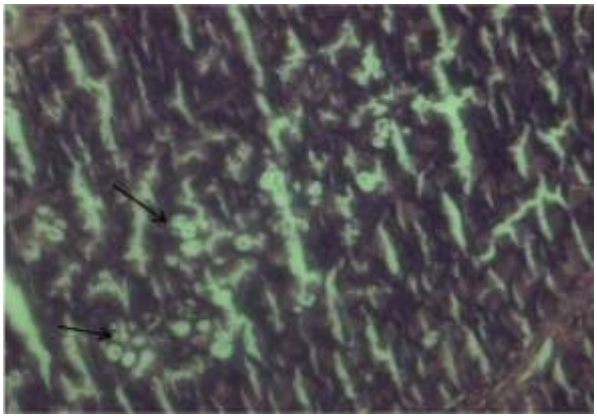


d. Methanolic leaf extract (200mg/kg b.w) treated rats: Glandular pancreas with islets cells normal, Non glandular region normal (white arrow), Ductular epithelial cells hyperplasia and periductular fibrosis noticed (red arrow)



e. Methanolic leaf extract (400mg/kg b.w.) treated rats: Mild vacoular/ fatty degeneration pancreatic acini located in the non glandular region (Black arrow)

IV. DISCUSSION

Streptozotocin (STZ) induces diabetes mellitus by causing selective cytotoxicity on pancreatic islet β-cells through the release of nitric oxide radical. It ultimately results in β-cells necrosis. The STZ induced diabetic rats exhibited elevated serum glucose, cholesterol, LDL protein, urea, uric acid and creatinine. Further, the marker enzymes of diabetic liver viz., Aspartate aminotransferase (ALT) and Alanine aminotransferase (ALP) were also found in elevated level. The antioxidant enzymes (superoxide dismutase, catalase, reduced glutathione) were greatly affected by diabetes and significantly decreased in diabetic rats.

Glebenclamide an antidiabetic drug usually stimulates insulin secretion from pancreatic β-cells by inhibiting ATP sensitive K-ATP channels in plasma membrane. The diabetic rats administred with glebenclamide (60mg/kg) showed hypoglycemic effects through reduction in the elevated ALT and ALP marker enzymes along with decrease in serum cholesterol, triglycerides, urea, uric acid,creatinine and LDL protein. The antidiabetic activity observed in terms of lowering the level of above parameters is statistically significant.

The administration of D.ferrea leaf extracts (200mg and 400mg/kg) to diabetic rats also resulted in hypoglycaemic activity roughly equal in magnitude with glebenclamide. The methanolic leaf extracts brought down the elevated levels of serum glucose, cholesterol, triglycerides, LDL protein creatinine, ALT and ALP enzymes in diabetic rats. But this effect is more in the case of 400mg/kg extract than it was observed in 200mg/kg leaf extract treatment. The effects of extracts are stastically significant when compared with diabetic control.

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V. CONCLUSION:

Histopathological examination of diabetic rat liver showed foci of inflammation and necrosis of cells. The pancreas also showed cystic degeneration of islet cells and fibrosis. The administration of methanolic extract (400mg) of D.ferrea leaf to diabetic rats resulted in the regeneration of normal liver cells and islet cells in pancreas. These effects are comparable with antidiabetic effects of glebenclamide and also statistically significant when compared with diabetic control.

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